

INFLUENCE OF DEFOLIATION ON TOXIC ALKALOID CONCENTRATION AND ALKALOID POOLS IN TALL LARKSPUR

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Abstract—This study was replicated at two locations in the mountains of central Utah. In 1997, ten uniform plants of tall larkspur (*Delphinium barbeyi*) in the early bud stage (40 cm in height) were selected at each site and clipped at 5 cm above soil level. In 1998, one stalk from each plant was harvested on a weekly basis; in 1999, one stalk was harvested at four times during its phenological development. Toxic and total alkaloid concentrations were measured and alkaloid pools in the entire stalk were calculated. Clipping reduced stalk height to less than 50 cm in 1998 and 65 cm in 1999, compared to over 100 cm in unclipped control plants. Alkaloid concentration was similar to control plants, but toxic alkaloid pools were 70% lower than control plants, because of the reduction in biomass of the stalks. Clipping reduced subsequent vigor and the amount of toxic and total alkaloids in tall larkspur.

Key Words—Tall larkspur, *Delphinium barbeyi*, norditerpenoid alkaloids, defoliation, cattle poisoning.

INTRODUCTION

Larkspurs (*Delphinium* spp.) are an important group of poisonous plants on mountain rangelands. They are relatively palatable to all livestock (Pfister et al., 1988) but acutely toxic to cattle (Olsen, 1978), causing widespread deaths throughout the mountains in the western United States (Nielsen and Ralphs, 1988).

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The toxic compounds in larkspur are norditerpenoid alkaloids. Alkaloids containing the *N*-(methylsuccinimido)-anthranilic ester group (referred to as MSAL alkaloids) are the most toxic (Manners et al., 1995), with methyllycaconitine (MLA) being the most prominent toxic alkaloid. We have differentiated the class of toxic alkaloids (MSAL) in larkspurs from the other alkaloids (Gardner et al., 1997) and quantified them as a measure of potential toxicity to cattle (Ralphs et al., 1997).

Environmental stresses on tall larkspur (*D. barbeyi*) have little effect on toxic alkaloid levels. Light stress and photosynthesis inhibition (Ralphs et al., 1998a) and the herbicide glyphosate (Ralphs et al., 1998b) reduced the dry weight of the plants, thus increasing alkaloid concentration proportionately, but the absolute amount of alkaloids, or alkaloid pools, were not affected. Damage from the larkspur mirid (*Hopplomachus affiguratus*) actually reduced toxic alkaloid concentration (Ralphs et al., 1998c), rather than inducing alkaloid synthesis, as suggested by the plant defense theory (Tallamy and Raupp, 1991).

Laycock (1975) clipped Duncceap larkspur (*D. occidentale*) in the vegetative stage for two successive years and reported that the concentration of total alkaloids declined by 50%, compared with unclipped plants. If defoliation reduced the toxicity of larkspur, clipping or mowing larkspur patches might reduce the risk of poisoning cattle. However, the generalized plant defense theory (Rhoades, 1979, 1985) predicts that some plants induce synthesis of toxic defense compounds in response to defoliation as a self-defense mechanism. Sometimes the response is delayed to the next season's growth (Tuomi et al., 1990). This delayed resistance may be an active response induced by defoliation (Haukioja, 1980) or an indirect response resulting from nutrient stress (Tuomi et al., 1990; Bryant et al., 1993). The carbon/nutrient balance theory predicts that if either carbon or nitrogen limits growth, the other nutrient becomes excessive and can be shunted to defense compounds (Bryant et al., 1983). The objective of this study was to determine the effects of clipping tall larkspur on subsequent vigor, alkaloid concentration, and alkaloid pools.

METHODS AND MATERIALS

The study was replicated at two locations in the mountains of central Utah. The Ferron Reservoir site is 46 km west of Ferron at an elevation of 3150 m. It is in a subalpine zone and the vegetation consisted of scattered subalpine fir stands interspersed in the tall forb plant community dominated by tall larkspur, western cone flower (*Rudbeckia occidentalis*), sweet cicely (*Osmorhiza occidentalis*), and mountain brome (*Bromus carinatus*).

The Mt. Terrell site is 40 km east of Salina at 3230 m in the subalpine zone. Tall larkspur dominated the tall forb community with violet (*Viola purpurea*),

sedge (*Carex* spp.), and mountain brome as understory species. Both sites were fenced to prevent any grazing interference during the experiment.

Ten uniform plants (20–30 stalks/plant) were selected at each site and marked with an orange tent stake. The plants were clipped 5 cm above the soil on July 16, at Salina and July 17, 1997 at Ferron. The plants were in the early bud stage, prior to elongation of the inflorescence. In 1998, one stalk was harvested from each plant, on a weekly basis, to evaluate seasonal trends in toxic and total alkaloid responses to clipping the previous year. In 1999, one stalk was harvested in the late vegetative, early flower, late flower, and pod stages of development. The height of the stalk was measured when harvested. It was placed in an airtight plastic bag and immediately frozen on Dry Ice. The samples were freeze dried and then weighed to obtain dry weight. After grinding through a cyclone grinder, the alkaloids were extracted and analyzed by Fourier-transformed infrared spectroscopy (FTIR) (Gardner et al., 1997) to determine the concentration of the toxic MSAL alkaloids and total alkaloids. Alkaloid pools in each stalk were calculated by multiplying alkaloid concentration by the dry weight of the stalk (Ralphs et al., 2000). Pools are a better estimate of secondary compound synthesis because concentration can change by being diluted in a large plant, or concentrated in a small plant (Bryant et al., 1993).

The data were analyzed in two parts. The first analysis compared vigor measurements (number of stalks and dry weight per stalk) in the same plant when it was clipped in 1997 and at equivalent growth stages in 1998 and 1999. Data were analyzed in a random mixed analysis of variance in a split-plot design comparing locations and year, with plants within location as a random factor. The data were transformed by the log transformation prior to analysis, but actual means are presented. There was a location \times year interaction ($P < 0.01$), so the model was reduced to a one-way analysis of variance comparing years within each location. Means were separated by least significance difference pairwise comparisons.

The second analysis compared seasonal trends of stalk height and alkaloid concentrations and pools between the plants that had been defoliated in 1997, and 10 undefoliated control plants at each location in 1998 and 1999. These variables were compared in a repeated measures mixed analysis of variance using compound symmetry covariance in a split plot design. Treatment (control, clipped), location, and year were the main effects, and plants within treatment and location was the factor repeated over weeks. The data were transformed by log transformation prior to analysis, but actual means are presented in the graphs.

RESULTS

Vigor: There was an initial difference in the number of stalks per plant between the two locations ($P < 0.001$). The Salina site had an average of 31

TABLE 1. VIGOR OF TALL LARKSPUR WHEN CLIPPED IN 1997 IN EARLY BUD STAGE AND RESPONSE IN 1998 AND 1999^a

Location	Year	Plants (N)	Stalks/plant (N, mean \pm SE)	Stalk weight (g, mean \pm SE)
Ferron	1997	10	18 \pm 2.3 a	5.3 \pm 1.1 a
	1998	8	7 \pm 1.4 b	1.8 \pm 0.4 b
	1999	8	9 \pm 3.4 b	2.2 \pm 0.4 b
Salina	1997	10	31 \pm 1.7 a	3.6 \pm 0.3 a
	1998	9	16 \pm 2.5 b	1.7 \pm 0.2 b
	1999	8	32 \pm 4.9 a	1.5 \pm 0.1 b

^aMeans within locations not followed by the same letter differ ($P < 0.05$).

stalks/plant, compared with 18 stalks/plant at Ferron. Clipping greatly reduced the vigor of tall larkspur plants, in both number of stalks per plant and stalk weight (Table 1). The number of stalks per plant declined by 50% in 1998 and remained low in 1999 at Ferron. Stalks per plant increased, however, to pre-treatment levels in 1999 at Salina. Stalk weight declined at both locations and remained low in both years.

Figure 1 illustrates the growth pattern of larkspur plants through the 1998 growing season. The control plants exceed 100 cm in height when mature. The plants clipped in 1997 reached a height of only 50 cm in 1998 and 65 cm in 1999 ($P < 0.001$).

Alkaloids. The concentrations of toxic and total alkaloids in defoliated plants were similar to the control plants and steadily declined through the growing season in both years (Figure 2). There was a year difference ($P < 0.001$) with both toxic and total alkaloids slightly lower in 1999.

There was considerable variability in concentration of toxic alkaloids in defoliated plants in 1998 (i.e., wide standard error bars; Figure 2). Some of the defoliated plants were severely stunted. Their stalks were small and spindly, and they remained in the vegetative stage throughout the growing season. In some but not all of these stunted plants, toxic and total alkaloids remained high throughout the growing season, in contrast with the other plants in which alkaloid concentrations declined with maturity. Perhaps the prolonged juvenile stage of the stunted plants accounted for the elevated and retained alkaloid concentrations.

The total amount of alkaloid in a stalk, or alkaloid pool, regardless of its size, was estimated by multiplying the dry weight of the stalk by its alkaloid concentration. Pools of both toxic and total alkaloids in clipped plants were only one third of the undefoliated control plants (Figure 3). Clipping suppressed both the growth and total amount of alkaloids in tall larkspur in the subsequent growing season.

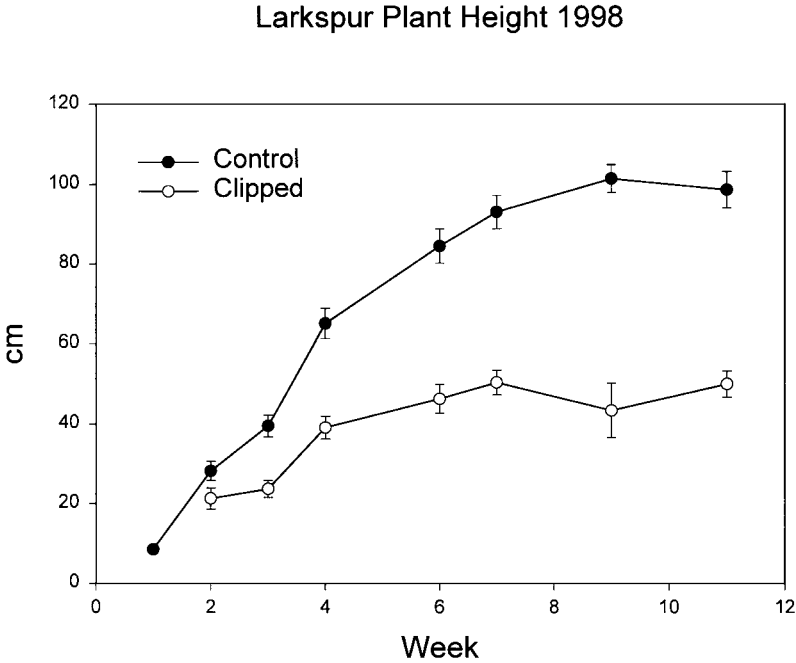


FIG. 1. Tall larkspur plant height through the 1998 growing season of undefoliated control plants and plants that were clipped in 1997.

DISCUSSION

Clipping tall larkspur in the early bud stage in 1997 greatly reduced the vigor of the same plants in 1998 and 1999. This agrees with the results of Laycock's (1975) experiments in which clipping duncicap larkspur in the early vegetative stage for two years was most detrimental to vigor when measured in the third year. Cronin (1971), however, reported that clipping tall larkspur at various frequencies during the growing season was not detrimental to the growth of the same plants the following year, but they failed to produce flowers. Vigor of other forbs in the tall forb community is also reduced by clipping: bluebell (*Mertensia arizonica* var. *leonardi*) (Laycock and Conrad, 1969), white polemonium (*Polemonium foliosissimum*) (McDonough and Laycock, 1975), Porter ligusticum (*Ligusticum porteri*), edible valerian (*Valeriana edulis*), and Richardson geranium (*Geranium richardsoni*) (Julander, 1968).

Laycock (1975) further reported that the concentration of total alkaloids declined by 50% following two years of clipping. Earlier extraction methods for the titration analytical technique (Williams and Cronin, 1963) did not retrieve

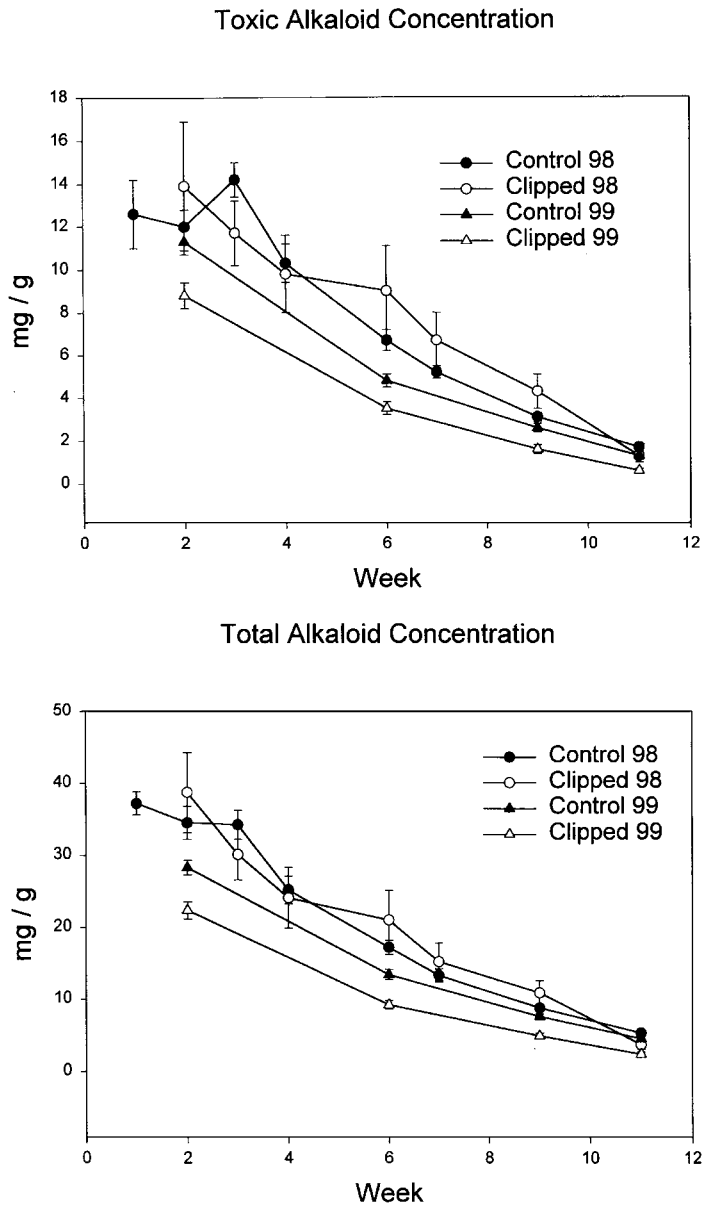


FIG. 2. Trends in toxic and total alkaloid concentration through the 1998 and 1999 growing season of control plants and plants that were clipped in 1997.

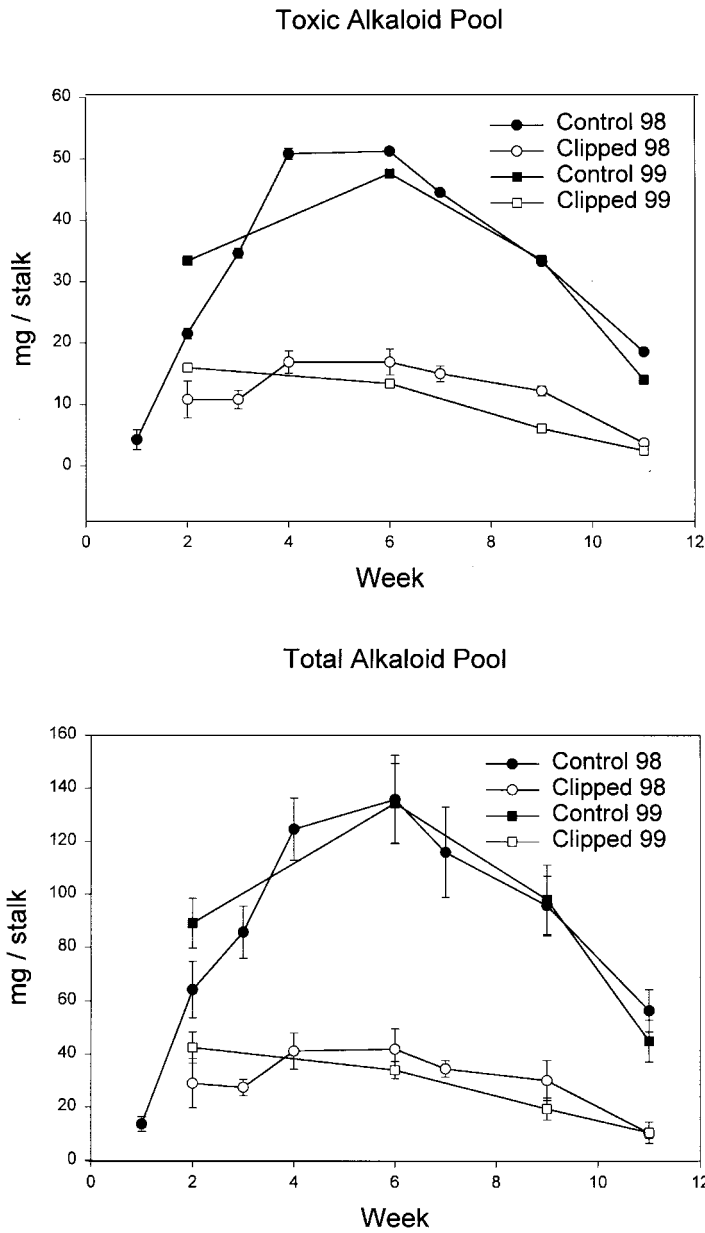


FIG. 3. Pools of toxic and total alkaloids in control plants and clipped plants in 1998 and 1999.

the toxic class of alkaloids (G. D. Manners, personal communication) and would subsequently underestimate the total alkaloid concentration. However, this does not explain the relative decline in total alkaloid concentration in Laycock's (1975) study and the lack of decline in alkaloid concentration in our study.

There was very little regrowth of clipped plants in 1997, and they were substantially smaller in 1998, both in height and stalk weight. Clearly their photosynthetic capacity was reduced. The carbon–nutrient balance theory (Bryant et al., 1983, 1992; Toumi et al., 1990) predicts that since carbon gain would be limited, nitrogen in excess of that used for growth could be used to increase synthesis of alkaloids. However, alkaloid synthesis apparently did not increase (alkaloid concentration was similar to controls and alkaloid pools were lower). In contrast, wild tobacco shifts its allocation of nitrogen to nicotine synthesis following clipping, at the expense of growth and reproduction (Ohnmeis and Baldwin, 1994; Baldwin et al., 1990).

Larkspur grows from buds on the crown of the tap root. These buds start to swell in the late summer and early fall, remain active and grow slowly beneath the snowpack over winter, and initiate rapid growth even before the snow melts in the spring (Kreps, 1969). Perhaps the removal of photosynthetic material during the 1997 growing season greatly restricted the energy flow to the crown and developing buds, resulting in the significant reduction in the number and vigor of stalks in 1998 and 1999.

Benn and May (1964) reported that alkaloids in *Delphinium brownii* are synthesized in roots and probably follow the mevalonate pathway of terpene biosynthesis or the recently proposed pathway starting from pyruvate and glyceraldehyde-3-phosphate (Lichtenthaler et al., 1997). Since energy and carbon were apparently limited in the roots of our clipped plants, alkaloid biogenesis may also have been limited.

Tall larkspur is not palatable to cattle in its early growth stages, but becomes palatable as it matures into the flower and pod reproductive stages (Pfister et al., 1997). Since clipping reduced the number and size of larkspur stalks, and prevented them from maturing into the reproductive stages, it may reduce the likelihood of cattle grazing clipped plants, as well as reducing the total larkspur biomass available to grazing animals. Alkaloid pools were also lower in the shorter stalks, although concentration of alkaloids remained unchanged. The reduced vigor of clipped plants may shift the competitive advantage to associated species in the plant community, which could further suppress larkspur growth. Thus, clipping may reduce the overall risk of poisoning in cattle.

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